REMARKS

Claims 1-13 currently appear in this application. The Office Action of February 22, 2007, has been carefully studied. These claims define novel and unobvious subject matter under Sections 102 and 103 of 35 U.S.C., and therefore should be allowed. Applicant respectfully requests favorable reconsideration, entry of the present amendment, and formal allowance of the claims.

Rejections under 35 U.S.C. 112

Claim 1 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The phrase "highly compressed CO_2" is said to render the claim indefinite because it is unclear whether the limitations following the phrase are part of the claimed invention. Claim 1 is also said to conflict with claim 3, since claim 1 recites "highly compressed CO_2" and claim 3 does not.

This rejection is respectfully traversed. Claim 1 has been amended to recite that the extraction is performed at pressures above 500 bar. Claim 3 has been amended to recite "highly compressed CO_2." In both claim 1 and claim 3, "highly compressed CO_2" means that the pressures of the extraction

occurs at pressures above 500 bar (claim 1) or at pressures of 600 to 1000 bar (claim 3). Since the "high pressure" is defined in the claims, it is respectfully submitted that this is not indefinite.

Art Rejections

Claims 1, 2 and 8 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kuhrts, US 2003/0228369 in view of Pilz et al., US 4,263,253. The Examiner concedes that Kuhrts is silent with respect to pressures and temperature for the extraction processes. Pilz is said to teach dissolving a solid in a gas which is under supercritical conditions of temperature and pressure at pressure between 20 and 1200 bar.

This rejection is respectfully traversed. The method claimed herein produces a xanthohumol-concentrated hop extract, that is, a hop extract having up to 40% xanthohumol (Example 4, page 7). Kuhrts discloses no pressures and temperatures for extracting alpha acids from hops using supercritical CO_2, and does not indicate that the extract contains high percentages of xanthohumol, only alpha acids.

Kuhrts discloses the use of supercritical CO_2 to extract hop pellets, which was disclosed in the specification as filed at page 4, line 11 to page 5, line 7. As a summary of these comments, it should be emphasized that prior to the present invention, one skilled in the art believed that

optimum extraction conditions for hops were 200-300 bar and 40-60°C, with a maximum of 500 bar. Therefore, one skilled in the art would assume that Kuhrts extracted alpha acids from hops using pressures at about 300 to 500 bar.

In addition, the information in Kuhrts regarding the high concentration of alpha-acids (paragraph 0047) and that one of the primary alpha acids would be xanthohumol (paragraph 0045) is not correct. Only humulon is an alpha acid, whereas xanthohumol is a polyphenol. As evidence of this, submitted herewith is a partial copy of "Manual of good Practice—Hop and Hop Products" issued by the European Brewery Convention. From page 26, paragraph 4.1.2, we learn that humulone is the major component of alpha acids, whereas xanthohumol is listed under the phenolic acids on page 34, line 3 in paragraph 4.1.4. therefore, the disclosure of Kuhrts that extracting hops with supercritical CO_2 yields a high concentration of alpha acids is not relevant to the presently claimed method.

Pilz adds nothing to Kuhrts, because Pilz discloses dissolving solids in a supercritical gas and filtering the solution through a sterile filter to render the solid sterile. Examples of suitable solids are analgesics such as aspirin and antibiotics such as ampicillin and azlocillin. Pilz gives a broad range of pressures and temperatures for this process, and there is no indication that this process is used for

separating xanthohumol from hop extracts. The Pilz process depends upon the solubility of the solid in the supercritical gas, which may be other than CO_2 or a mixture of CO_2 with other gases. One skilled in the art reading Kuhrts with Pilz would not be motivated to raise the pressure above about 500 bar to extract xanthohumol from hop extract.

Pilz discloses at column 2, lines 2-5, that German patent 21 27 618 describes a process for preparing hop extracts. This is the same patent cited in the present specification at page 4, line 13, wherein the pressure is 200-400 bar and the temperature is 45-50°C. These are the prior art conditions, and do not even suggest the pressure and temperature ranges claimed herein. Pilz leads away from the presently claimed method, because Pilz cites conventional conditions for hop extraction with supercritical CO_2.

Claims 3-7 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kuhrts in view of Pilz and Edelmeier et al., US 2005/0042318 and Babish et al., US 2003/0113393.

This rejection is respectfully traversed. First of all, Kuhrts does not obtain an extract that is particularly rich in xanthohumol, but only alpha acids in an amount of 40-90%. In contrast thereto, what is claimed herein is obtaining high concentrations specifically of xanthohumol, not alpha acids in general. Pilz is silent with respect to extracting

xanthohumol or any alpha acids from hops, so Pilz adds nothing to Kuhrts.

Babish merely discloses removal of the solvent, but is silent with respect to obtaining xanthohumol *per se*. In fact, Babish discloses at paragraph 0040 that the extracts obtained have an alpha-acid content of greater than 45 percent by weight and, beta acid content greater than 45 percent by weight. There is no guidance on obtaining only xanthohumol, as claimed herein.

Erdelmeier et al. merely disclose that it was well known in the art to carry out several extractions of hop material using supercritical carbon dioxide to increase the content of xanthohumol. It should be noted that Erdelmeier disclose at paragraph 0016 that hop is treated to remove lipophilic and hydrophilic fibers and that extraction of xanthohumol depends on the temperature of preextraction with water. This certainly would not lead one skilled in the art to extract xanthohumol with carbon dioxide at the pressures claimed, whether the extraction was conducted once or several times.

In addition, claim 3 recites a pre-extraction with lower pressure (200-300 bar) and lower temperatures (40-60°C), followed by subsequent extraction with pressures of 600-1000 bar and temperatures of 60-90°C. However, Erdelmeier only

discloses extraction conditions of 250 bar and 50°C (see paragraphs 0040, 0042 and 0044). Again, Erdelmeier leads one skilled in the art away from the high pressure/high temperature extraction with supercritical CO_2 as claimed herein. Moreover, Erdelmeier is silent with respect to a pre-extraction at lower temperatures and pressures followed by a subsequent main extraction using higher temperatures and pressures.

Claims 9-13 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kuhrts in view of Pilz and Ohnogi et al., US 2004/0002423.

This rejection is respectfully traversed. Ohnogi discloses that it is well known to add ethanol based hop extracts to food and beverages. However, these extracts are not the same as those obtain by the herein claimed process. It has been demonstrated that the specific pressures and temperatures used in the claimed process produce enhanced yields of xanthohumol. There is nothing in Kuhrts or Pilz that would lead one skilled in the art to obtain xanthohumol extracts using the conditions recited in the claims. Therefore, the food prepared using xanthohumol as obtained by the herein claimed process is not the same as food prepared using an ethanol extract derived from hops. While ethanol may be used as an appropriate solvent, the extracts obtained by

Kuhrts or Ohnogi are not the same as the xanthohumol extracts obtained by the method claimed herein.

In view of the above, it is respectfully submitted that the claims are now in condition for allowance, and favorable action thereon is earnestly solicited.

Respectfully submitted,

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From "Manual of Good Practice – Hop and Hop Products", published by the European Brewery Convention

4. Hop Chemistry

4.1 CHEMICAL COMPOSITION OF HOPS

4.1.1 Secondary Metabolites in Hops

The term 'secondary metabolites' is used to denote substances that are formed in plants but do not participate in primary metabolic processes, but which are necessary for life and development of the plant. They are regarded as being an essential part of the intricate system used by plants in the battle to survive and propagate. Their role may centre on defence of the plant against predators, pathogens or competitors, on aid to pollination or seed dispersal, on protection against or adaptation to extrinsic abiotic factors, or on a combination of these functions. Most of these compounds through interaction with the environment enhance the prospects for survival of the plant or its offspring.

The multiplicity of the chemical structures of secondary products is enormous. Thousands of secondary metabolites have now been identified and the number is rapidly increasing with the investigation of a growing number of plants. While animals eliminate unwanted metabolic products, plants store most secondary metabolites. The retention in the plant body facilitates their further transformation, while it is one of the reasons for the multiplicity of secondary products.

The secondary metabolites may be derived from components of any of the vital biochemical pathways or may simply be waste products modified to serve a useful purpose. Most reactions in secondary metabolic pathways are catalysed by specific enzymes, hence they are not to be considered as side products of primary metabolism. The more chemical reactions necessary for the biosynthesis of a given secondary substance, the more restricted is its distribution. Many plant constituents are bioactive or even toxic; they may have applications, e.g. in medicine or in cosmetics, but this is not a prerequisite for usefulness within the plant.

Hops contain hundreds of secondary metabolites comprising many different groups of organic compounds, (see Table 2.1). Of particular interest are the so-called resins (containing mainly hop acids), hop oil and polyphenols. These three classes are important as biochemical markers to differentiate hop varieties. For this purpose, variances in the content of individual components and the absence or presence of particular components can be exploited. The varying physiological properties (sedative, oestrogenic, hypnogenic, bacteriostatic, etc.) of hops have been known for many centuries and in the plant sections of most Pharmacopoeia hops occupy a pre-eminent position.

It has not been possible, to date, to detect in the polyphenol fraction and in the hop oils, compounds not occurring elsewhere in the plant kingdom. On the other hand, the hop acids have hitherto not been found in any other plant species and have thus chemotaxonomic value, i.e. for classification of plant

species based on the chemical content. During evolution, hops must have developed a very special defence mechanism and the hop acids may well play a crucial role in this respect. An intriguing feature of the hop acids is their exceptionally high content, up to 25% or even more, of the dry weight of the hop cones. Usually, secondary metabolites are toxic for the plant itself, hence they are compartmentalised, i.e. stored in particular vacuoles in the cell. Therefore, the content is very low, as it is for typical secondary metabolites such as alkaloids. Apparently, the hop plant has dealt with this phenomenon by excreting the hop acids in exterior organs, the so-called lupulin glands, (see pages 23 and 24). Since the incentive for the synthesis of hop acids *in vivo* is not known, attempts to produce them in artificial media, e.g. cell suspension cultures, have failed.

4.1.2 Hop Resins

In general, hop resins comprise non-specific fractions and specific constituents or mixtures thereof. Soft resins are soluble in hexane, while hard resins are insoluble. The combined soft and hard resins form the total resins, which are soluble in cold methanol and diethyl ether. The provision that the total resins should be soluble in cold methanol is included to distinguish between resins and wax. Hop wax, a mixture of long-chain alcohols, acids, esters and hydrocarbons, is very poorly soluble in cold methanol.

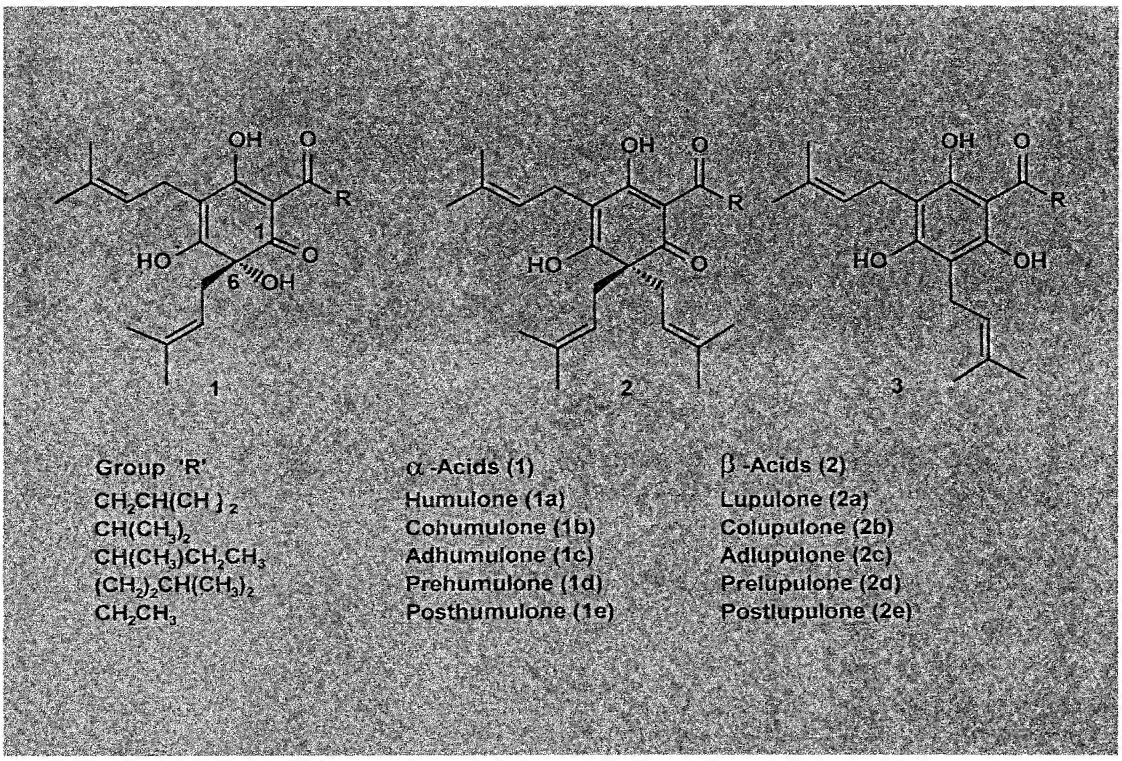
The hop acids, part of the soft resin fraction, consist of two related series, the α-acids (because they were discovered first) and β-acids, respectively (for a review, see Verzele & De Keukeleire, 1991). These compounds occurring as pale-yellowish solids in the pure state, are weak acids, exhibit very poor solubility in water and have almost no bitter taste. Most important are the α–acids or humulones. To date, 5 analogues of the α–acids (isomers and homologues) have been characterised, i.e. humulone (**1a**), cohumulone (**1b**), adhumulone (**1c**), prehumulone (**1d**) and posthumulone (**1e**), (see Scheme 4.1.1).

The major component of the α–acids mixture, humulone, is also known in the literature as n-humulone or 'normal' humulone, but the use of this notation should be discouraged since in IUPAC (International Union of Pure and Applied Chemistry) nomenclature *'n'* or *'normal'* refers specifically to a straight carbon chain. The relative amount of the adhumulones within the α-acids is fairly constant between varieties (ca. 15%), while the relative amounts of humulone and cohumulone are variety-dependent (20-50%). Cohumulone has been associated with a poor hop quality, although this issue is not proven unambiguously. Pre- and posthumulone are minor constituents. Detailed analysis by HPLC (high performance liquid chromatography) - MS (mass spectroscopy) reveals the presence of yet other related compounds in small concentrations.

The β–acids or lupulones also comprise 5 analogues, corresponding to those of the α–acids, namely lupulone (**2a**), colupulone (**2b**), adlupulone (**2c**), and the less important prelupulone (**2d**) and postlupulone (**2e**), (see Scheme 4.1.1). Both series of hop acids appear to have common biochemical precursors, the 6-deoxy-α-acids (**3**). However, the relative proportions of α-acids and β-acids, as well as the content of co-homologues, depend strongly on the hop variety and, for a given variety, on the conditions of growing.

In the internationally approved HPLC analytical methods the α-acids are separated from the β-acids and the co-derivatives from the other homologues. Consequently, the 6 major hop acids are usually

Scheme 4.1.1 Chemical structures of the α-acids, the β-acids and the 6-deoxy-α-acids

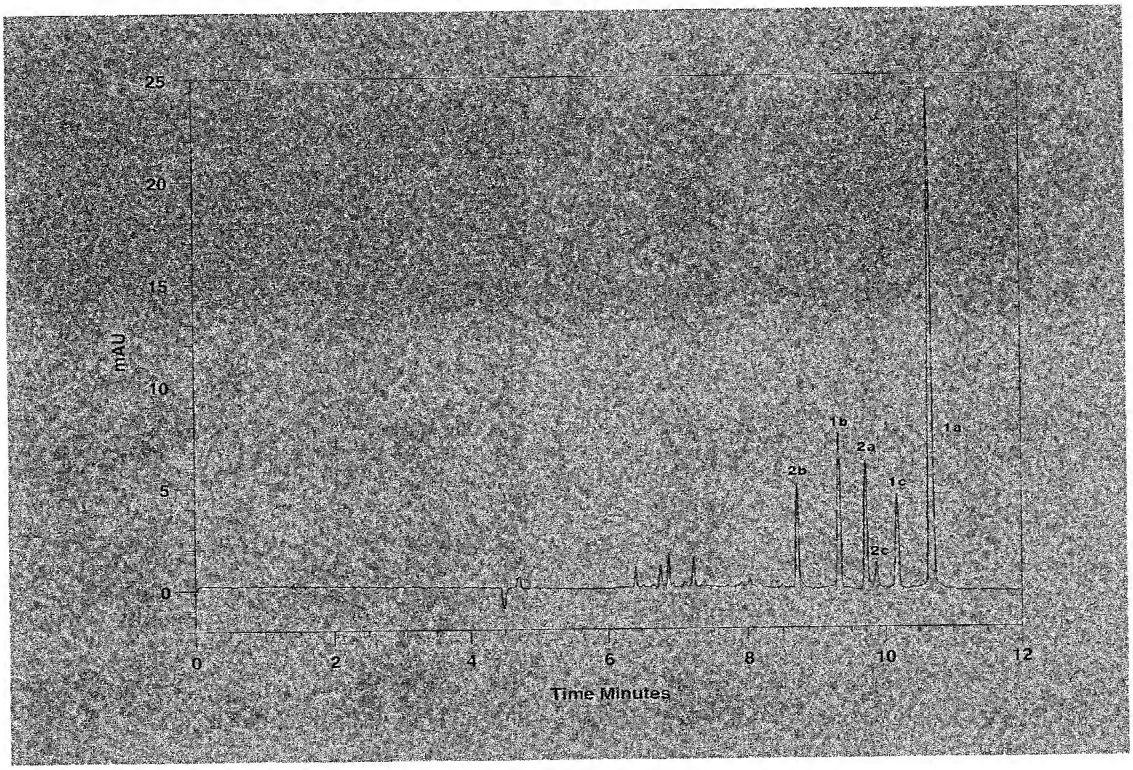


resolved into 4 peaks, (see Section 7.1.7). Separation of the individual hop acids is possible when using appropriate HPLC conditions. Alternatively, full separation, identification and quantification is achieved in a short analysis time by MEEKC (microemulsion electrokinetic chromatography) with DAD (diode array detection). This technique can be applied to whole hops in a fully automated sequence involving SFE (supercritical fluid extraction at a high density of carbon dioxide) - MEEKC - DAD (Sandra et al., 1996). By using two-step SFE the hop acid analysis can be combined with analysis of the hop oil, thereby providing complementary information for identification of hop cultivars and hop quality control, (see Section 4.1.3).

Figure 4.1.1 shows the MEEKC separation of the hop acids in the hop variety Mt. Hood, in which the presence of an oxidised fraction eluting before the main compounds may be noted. Variations in relative abundances of this fraction reflect different degrees in oxidation due to unsatisfactory drying conditions during harvest or as a clue to the variety-dependent storage stability of hops and hop products (see Section 7.1.13). The bittering value of aged hops is less than that of fresh hops due to gradual degradation of the α-acids. This decrease is only partially compensated for by an increase of bitter-tasting oxidised derivatives of the β-acids.

The hop acids have pronounced bacteriostatic activity; they strongly inhibit the growth of Gram-positive bacteria. This action has been attributed to the interference of the prenyl group

Figure 4.1.1 MEEKC analysis of the hop acids in the variety Mt. Hood (from Sandra et al., 1996)



1a: humulone; 1b: cohumulone; 1c: adhumulone;
2a: lupulone; 2b: colupulone; 2c: adlupulone

characteristic of the side chains of the hop acids with the function of the cell plasma membrane. It appears that the more prenyl groups (3 in the β-acids) are present, the stronger the bacteriostatic action.

4.1.3 Hop Oil

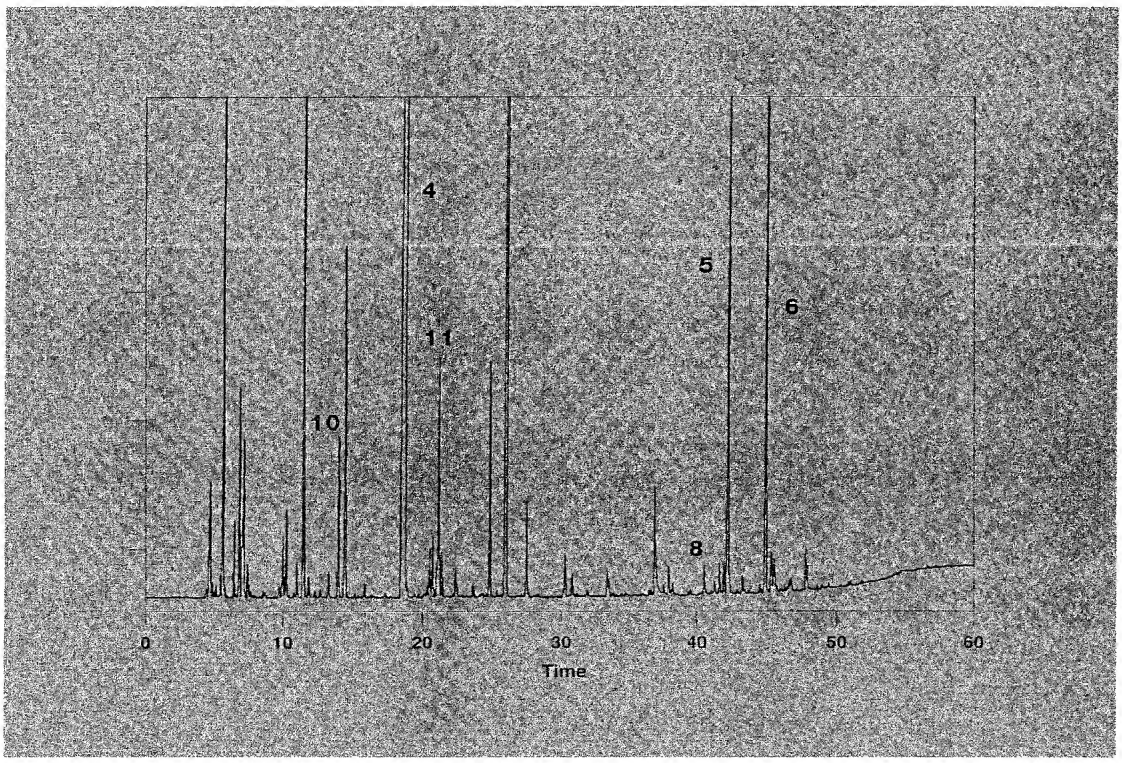
The hop oil represents a small, volatile fraction of hops (0.5-3.0%), in which over 300 components have been positively or tentatively identified (Moir, 1994). Current understanding is that the hop aroma is most likely the result of the interaction of many different constituents. The partition of hop oil components in different classes is represented in Table 4.1.1 without referring, however, to the varying respective proportions in the total oil (Sharpe & Laws, 1981).

Table 4.1.1 Composition of hop oil

Components	Approximate number
Esters	70
Hydrocarbons	60
Aldehydes - Ketones	60
Alcohols	50
Oxygen Heterocyclics	30
Sulphur-containing compounds	30
Carboxylic acids	10

The complex mixture of volatiles, occurring together with the hop acids in the lupulin glands, can be separated by CGC (capillary gas chromatography). Identification of individual components is achieved, either by the use of reference compounds, or, in a more comprehensive fashion, by coupling CGC with a mass spectrometer (MS). Hop oils can conveniently be profiled via head-space CGC, whereby the gas phase, in equilibrium with samples of whole hops, hop pellets or hop extracts in a closed vessel, is directly injected into the chromatograph, (see Section 7.2.7). Figure 4.1.2 shows a head-space chromatogram of a supercritical carbon dioxide extract of the hop variety Hallertau Perle.

Figure 4.1.2 Headspace CGC analysis of a supercritical carbon dioxide extract of the hop variety Perle



4: myrcene; 5: caryophyllene; 6: humulene; 8: linalool; 10: 2-methylpropyl isobutyrate; 11: 2-methylbutyl isobutyrate

Hop oils can be divided into an apolar (hydrocarbon) fraction (40 to 80%) and a polar (oxygenated and sulphur-containing) fraction, e.g. by solid-phase extraction (SPE) on silica, followed by CGC - MS. Sandra et al. (1996) have developed a fully automated analysis of the hop oils by SFE-SPE-CGC-MS (SFE: supercritical fluid extraction at a low density of carbon dioxide). In Figure 4.1.3 the CGC chromatograms of the respective apolar and polar fractions of the hop oil of the variety Nugget are displayed.

The hydrocarbon fraction consists mainly of the monoterpene myrcene (4) and the sesquiterpenes caryophyllene (5), humulene (6) and in some cases farnesene (7), (see Scheme 4.1.2). Many high-valued hops have significant percentages of humulene and low myrcene and caryophyllene contents. Humulene is thought to be partly responsible for the pleasant hop aroma, although this may have no impact on beer as this compound is readily oxidised. The presence or absence of farnesene has been used as a quality criterion. Myrcene and the sesquiterpene hydrocarbons (at least 40 members have been detected) are notoriously reactive. Oxidative processes followed by rearrangements give rise to a number of derivatives, including tricyclic sesquiterpenes, which may be of interest for the differentiation of hop varieties.

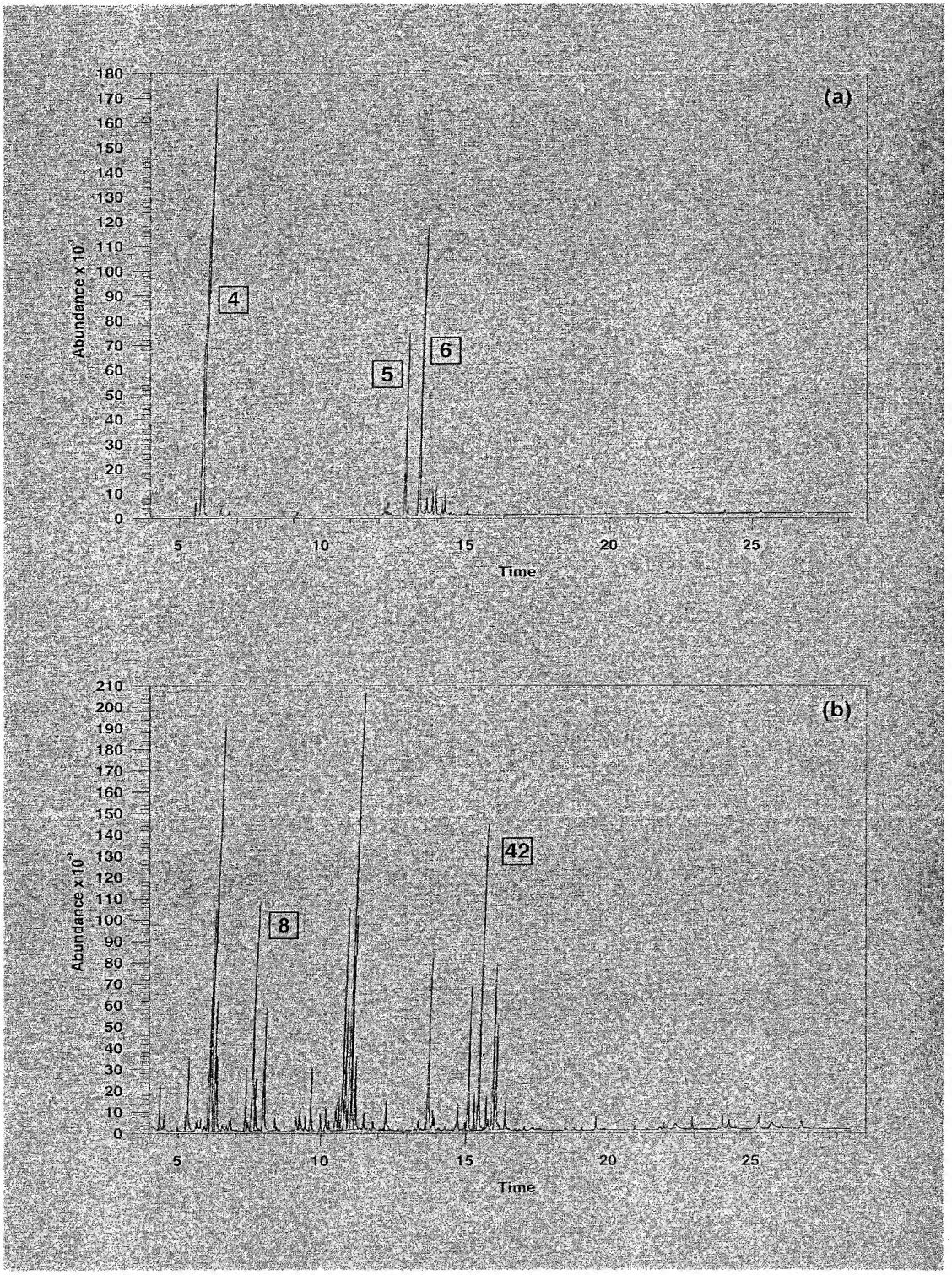
Linalool (8), the major monoterpene alcohol found in hops, and geraniol (9) are important oxygenated terpenes known for their floral scents. Many esters, such as 2-methylpropyl isobutyrate (10) and 2-methylbutyl isobutyrate (11), convey fruity aromas to hops. Fatty acids, e.g. 2-methylbutyric acid (12), characterise the cheesy aroma of old hops. Hop oils often contain a range of organosulphur compounds, which may have an adverse effect on beer flavour. These include thiols, sulphides, polysulphides, thioesters, thiophenes and episulphides, such as 1,2-epithiohumulene (13) and 4,5-epithiocaryophyllene (14). Although sulphur compounds are present in very low quantities in hops, some have flavour thresholds of a few parts per billion (ppb, microgram/kilogram) or even lower. Presently, the relative influence of individual sulphur compounds is still speculative. Hop oils show antimicrobial activity, especially against Gram-positive bacteria. The amount of these constituents, and particularly the ratios between them, can be used as clear varietal characteristics.

4.1.4 Hop Polyphenols

About 70-80% of the polyphenols in wort are derived from malt and only 20-30% from hops. It is likely that malt-derived and hop-derived polyphenols are different. Polyphenols represent a vast class of compounds with widely varying structural characteristics, but having in common the presence of one or more phenolic functional groups. It is a generally accepted fact that polyphenols have a quite significant influence on beer. The properties depend very much on the degree of polymerisation. Low-molecular-weight polyphenols are natural anti-oxidants and account to a great extent for the reducing power of wort, thereby protecting beer against oxidation and improving the taste stability. Higher-molecular-weight polyphenols contribute to the colour of beer and to haze formation. On the other hand, polyphenols may cause an unpleasant astringency.

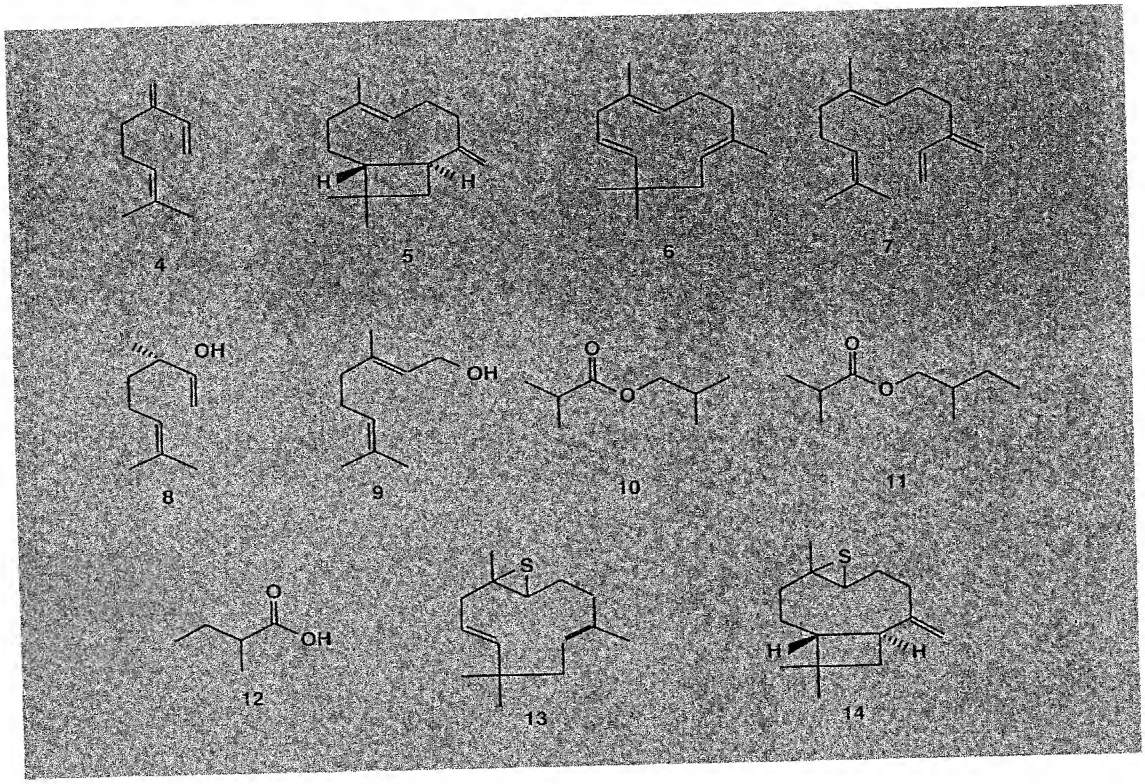
A well-established property of some polyphenols is their propensity to form stable, insoluble complexes with proteins that precipitate during brewing. In this respect, proanthocyanidins, e.g. proanthocyanidin B3 (15) composed of (+)-catechin (a flavanol) units (16) (see Scheme 4.1.2), are very active. These

Figure 4.1.3 CGC analysis of the apolar (a) and polar (b) fractions of the oil of the hop variety Nugget (from Sandra et al., 1996)



4: myrcene; 5: caryophyllene; 6: humulene; 8: linalool; 42: humuladienone

Scheme 4.1.2 Important constituents of hop oil



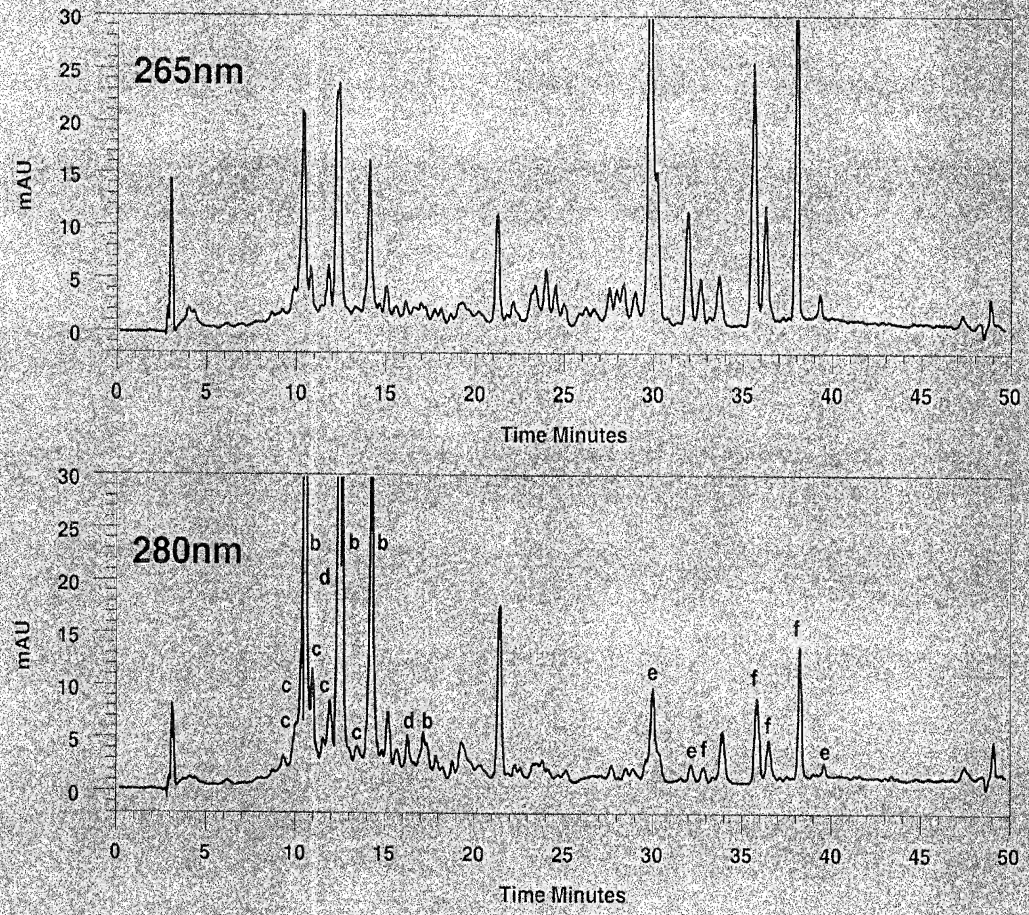
polyphenols are also known as tannoids, defined as low-molecular-weight polyphenols with a molecular mass between 500 and 3000, while anthocyanogens rather refer to glycosides of proanthocyanidins.

At least 100 components may be separated by means of HPLC-DAD as is highlighted for the hop variety Hersbruck Spät (see Figure 4.1.4) (Forster et al., 1995). Groups of substances that can be determined are given in Table 4.1.2, together with the concentration range in which these classes occur in hops. The composition of the polyphenols depends on the hop variety, cultivation area, harvesting technique and degree of ageing. Drying in air at 60°C or 80°C results in a drastic decrease of flavanols and proanthocyanidins.

Table 4.1.2 Groups of polyphenols in hops

Group	Concentration (mg kg^{-1})
Hydroxybenzoic acids (a)	< 100
Hydroxycinnamic acids (b)	100 - 300
Proanthocyanidins (c)	600 - 1,500
Flavanols (d)	300 - 1,100
Quercetin glycosides (e)	500 - 2,000
Kaempferol glycosides (f)	500 - 1,700
Flavonols (g)	< 100 - 200

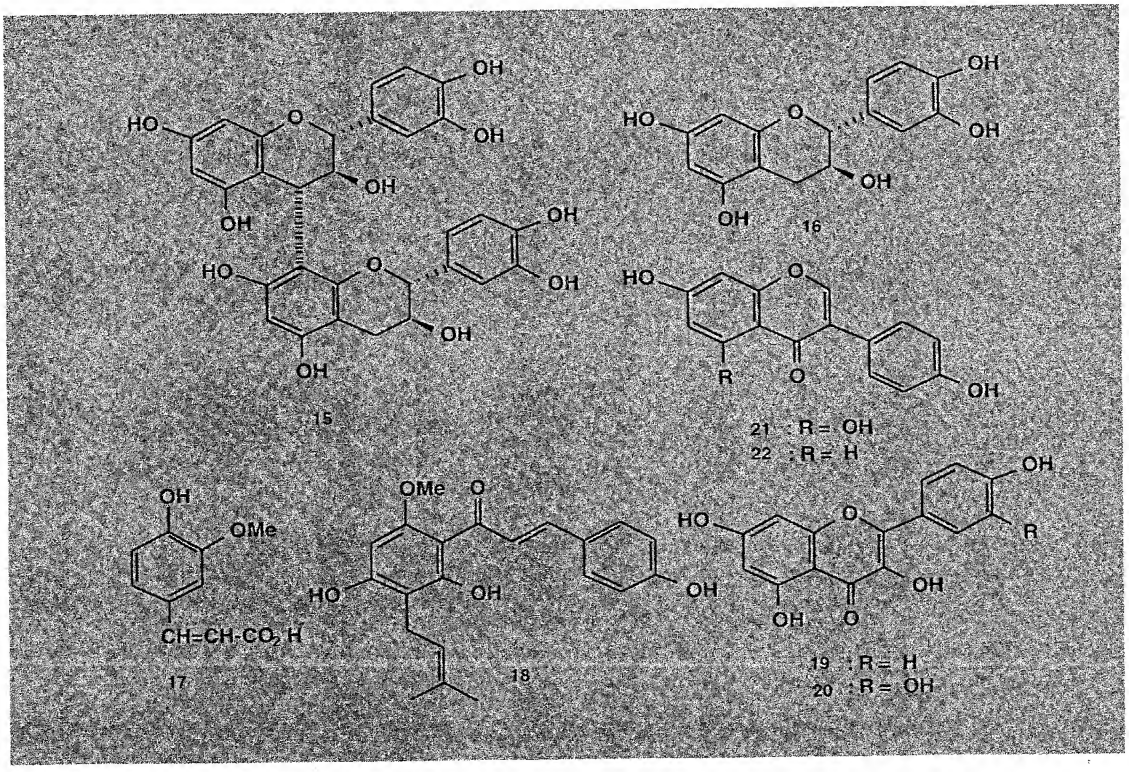
Figure 4.1.4 HPLC-DAD analysis of the polyphenols in the hop variety Hersbruck Spät (from Forster et al., 1995)



a: hydroxybenzoic acids (traces, not indicated); b: hydroxycinnnamic acids; c: proanthocyanidins; d: flavanols; e: quercetin glycosides; f: kaempferol glycosides; g: flavonols (traces, not indicated)

Phenolic acids are precursors for particular beer aromas; thus, ferulic acid (17) giving rise to 2-vinylguaiacol, contributes to the aroma of wheat beers. Other polyphenols include chalcones, e.g. xanthohumol (18), and flavonols, e.g. kaempferol (19) and quercetin (20). Most of these compounds occur to a great extent as glycosides and are partly transferred from hops to wort. Xanthohumol has been referred to as the 'hop hormone', although the oestrogenic action has never been proven unambiguously. Rather, isoflavones, e.g. genistein (21) and daidzein (22), or as yet unidentified polyphenols, may account for the weak oestrogenic properties of hops.

Scheme 4.1.3 Important hop polyphenols

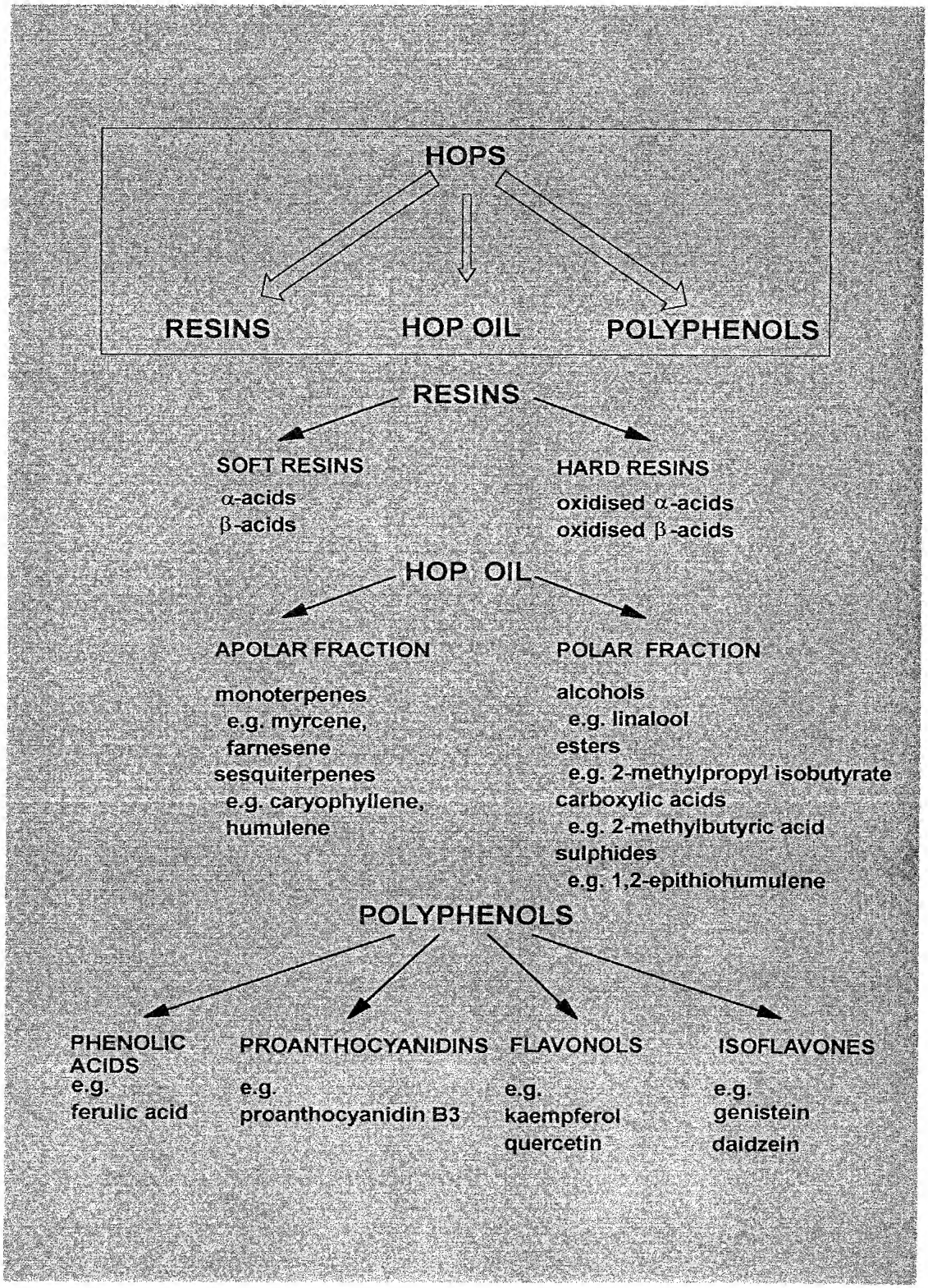


Scheme 4.1.4 gives an overview of the secondary metabolites in hops that are of importance in the brewing process. The three main classes, resins, oils and polyphenols, respectively, are further subdivided, while the most prominent specific constituents are indicated for each subclass.

4.1.5 The Oxidation State of Hops

The bitter substances in hops gradually degrade during storage. Other hop fractions, such as the hop oil and polyphenols, are also oxidised. Their oxidation products significantly affect beer flavour. Therefore, the oxidation state of hops, i.e. the degree of oxidative deterioration of hops during handling and storage, is an important quality factor. It is not possible to trace the fate of individual constituents as degradation pathways are multiple.

Scheme 4.1.4 Secondary metabolites in hops



The intervention of free radical reactions involving known active oxygen species, such as singlet oxygen, hydrogen peroxide, superoxide radical anions and hydroxyl radicals, or intermediate lipid oxidation products, is quite plausible. Oxidation is certainly a very important matter, which needs to be looked at more closely.